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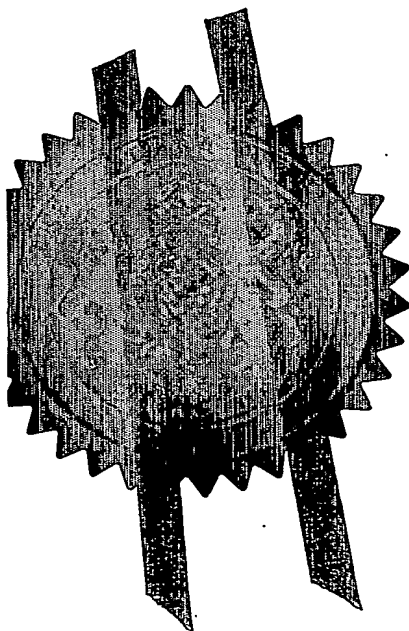
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GB 0216162.8

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of

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and

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Incorporated in the United Kingdom,

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THE PATENT OFFICE  
12 JUL 2002



12 JUL 02 E732331-3 002819  
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The Patent Office

Cardiff Road  
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1. Your reference

MJL/C1267/C

2. Patent number

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0216162.8

12 JUL 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Cytocell Limited  
Unit 6 Somerville Court  
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Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

SECTION 50 (1) ACT APPLICATION FILED 14/10/02  
6048755002

4. Title of the invention

Lateral Flow Assay Device and Method

5. Name of your agent (if you have one)

Keith W Nash & Co

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Patents ADP number (if you know it)

1206001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

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Claim(s)

Abstract

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Statement of inventorship and right to grant of a patent (Patents Form 7/77)

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I/We request the grant of a patent on the basis of this application

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Date 11 July 200

12. Name and daytime telephone number of person to contact in the United Kingdom

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**Title: Lateral Flow Assay Device and Method****Field of the Invention:**

The present invention relates to an assay device that may be used to perform an assay comprising nucleic acid isolation, nucleic acid stabilisation, specific sequence amplification and amplification product (amplicon) detection steps. The device is centred on lateral flow principles and the components of nucleic acid extraction, nucleic acid stabilisation, nucleic acid amplification and amplification product detection are preferably all located within the matrix of the device. In a preferred embodiment the present invention is concerned with the isolation, amplification and detection of specific nucleic acid sequences of interest, integrated into a single, disposable, easy to use, lateral flow device, and a method of performing an assay using the assay device.

**Background of the Invention**

Sensitive detection of nucleic acids has advanced over recent years with the development of a variety of nucleic acid detection and amplification techniques. These amplification techniques can be broadly divided into specific target amplification and signal amplification. Examples of target amplification techniques include, Polymerase Chain Reaction (PCR) (US 4,683,195 and US 4,683,202), Nucleic Acid Sequence Based Amplification (NASBA) (US 5,130,238), and Transcription Mediated Amplification (TMA) (US 5,399,491). Examples of signal amplification techniques include, Signal Mediated Amplification of RNA Technology (SMART) (WO 93/06240), Split Promoter Amplification Reaction (SPAR) (WO 99/37805), Invader (US 5,846,717) and Ligase Chain Reaction (LCR) (EP 0,320,308). These technologies can be further subdivided by their ability to amplify target / signal either by requirement for thermal cycling (such as PCR) or ability to operate at a single temperature (isothermally).

One commonality between these techniques is the requirement for front end sample preparation (nucleic acid extraction / isolation / purification) and back end amplicon detection. All of these techniques involve multi-step processes to achieve detection of the

amplicon, and current developments are targeted at complex systems for automation of these processes, to provide medium to high throughput of analytes.

There are a number of methods well described and illustrated in the prior art for the isolation and extraction of nucleic acids e.g. chaotropic agents, phenol/chloroform, chromatographic separation.

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One such technique utilises specially coated matrices, i.e. FTA technology (Whatman, US Patents 5,496,562; 5,807,527; 5,985,327; 5,756,126; 5,972,386; 5,985,327). The coating on the matrix (mainly cellulose-based) lyses cell and nuclear membranes, denatures proteins, and protects nucleic acid from UV and environmental damage. The base components of the coating are detergent, base, chelating agent and free radical trap. When samples are applied, membranes are lysed and nucleic acids released. Nucleic acid becomes immobilised within the matrix. Cellular impurities are washed away with FTA purification reagent leaving nucleic acids trapped within the matrix. Release of nucleic acids can be achieved by a number of means or amplification reactions achieved directly upon the matrix immobilised nucleic acids. The FTA technique has been used on a wide variety of sample types such as blood, tissue, cell lines, plant material, bacteria, viruses, hair root. The majority of biological samples are completely inactivated upon contact with the FTA matrix.

Nucleic acid amplification techniques have been well described and illustrated in the prior art and all rely on the action of nucleic acid dependent enzymes. One such technique, SMART (WO 93/06240) relies on the interaction of two probes combined with the sequence of interest to form a three way junction (TWJ) structure generating an RNA signal after the action of DNA polymerase and an RNA polymerase. The RNA signal generated from the TWJ may be further amplified by linear amplification probes, (WO 01/09376, Cytocell). Detection of the RNA signal may be achieved by a number of means that are well described in the *prior art* that include, but is not limited to, molecular beacons (US 5,925,517), latex bead / lateral flow, FRET / DFRET.

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For some of the aforementioned processes, expensive, complex equipment is required together with a level of skilled labour to perform such techniques. For these techniques to become widely used for both clinical and industrial applications, reduction in the complexity of the tests (i.e. number of steps and skill base required) together with a reduction in the instrumentation and cost per test are required.

Specifically, for these tests to be applicable at the near patient (point of care -PoC), or near process level, simple, easy to use, cost competitive systems are required.

Chromatographic or lateral flow assays have been used for many years to simplify the performance of tests such that they can be performed by semi- or unskilled users and require minimal equipment; they are therefore ideally suited to PoC tests. To date, however, their application has primarily been to immunoassays that are less complex than nucleic acid tests since they are simply detection assays, there being no amplification step.

The principle of lateral flow tests is a single, capillary device that contains some (or preferably all) of the reagents necessary for the performance of an assay. These reagents are typically contained within discrete zones of the device, such that as fluid flows along the device by capillary flow the various reactions occur sequentially and a signal is generated at a detection zone that is indicative of the amount of analyte in the sample. The devices can be a single membrane with reagents deposited at specific sites (e.g. US 4,161,146; US 4,361,537), or be composed of a series of discrete pads or membranes (each containing one or more reactants) arranged such that their edges are in fluid contact with one another (EP 0186799).

A typical lateral flow device will contain a sample receiving zone (which may contain buffers and chemicals necessary for the test), a label zone (which contains an analyte-binding reagent, such as an antibody, moveably immobilised in the pad, a capture and detection zone (which contains an analyte-binding reagent immovably immobilized on the membrane), and an absorption zone or sink of sufficient capacity to enable unbound labeled reagent to wash out of the detection zone. The pads and membranes are typically

attached to an impervious backing, and the pads and membranes are in fluid contact with one another (usually achieved by overlapping of the edges and use of adhesive or a lamination layer). Optionally the device is encased in a housing with defined apertures for sample application and visualization of result. Examples of such devices include those disclosed in US 5,656,503; US 5,622,871; US 5,602,040; US 4,861,711.

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To perform such an assay, sample is added to the sample application zone where it is drawn into the device by capillary force. Filter devices incorporated into the sample application zone can be used to remove blood cells, etc., and act as a volume control device (EP 0186799). The sample then hydrates and mixes with a labeled binding reagent (e.g. chromophore-labelled antibody), and any analyte present in the sample reacts with this to form a labeled analyte complex. This complex migrates along the device to the detection zone where a second binding reagent, immobilized on the strip, binds to the labeled analyte complex and prevents further migration of the labeled analyte complex. Unbound labeled binding reagent is drawn through the detection zone to the absorption zone. Thus, presence of signal at the detection zone is indicative of presence of analyte in the sample.

A variety of labels have been used for lateral flow assays, including radioactive (US 4,361,537) and fluorescent labels (US 6,238,931), visual labels are preferred for PoC applications. These include enzyme-generated colourimetric signals (US 4,740,468) and particulate chromophores (US 5,591,645; US 4,943,522; US 5,714,389).

Examples of fluid control systems used in immunoassay lateral flow assays include the use of chemical gates (US 6,271,040), centrifugal force (US 4,989,832), capillary restrictions (US 6,271,040), separate fluid channels of differing pathlengths for reagents (US 4,960,691; US 5,198,193), or physical means (e.g. the WheatRite test from C-Qentec).

The incorporation of enzyme systems within lateral flow assays has been reported, although they are concerned with generation or modulation of the final signal system

(rather than amplification of target or intermediate amplicon, as disclosed in the present invention). Examples include US 4,806,312; US 4,740,468).

The present invention provides an assay device which, in preferred embodiments, integrates nucleic acid extraction, specific sequence amplification and amplicon detection together with the incorporation of adequate controls for the prevention of false positive / negative results in a single easy to use and cost effective self contained lateral flow device.

Nucleic acids based analysis utilising lateral flow techniques has also been described in the prior art. For example, WO 00/12675 assigned to Molecular Innovations Inc., describes a system that integrates nucleic acid extraction, specific target amplification and detection. The device described therein comprises a hollow elongated cylinder with a single closed end and a plurality of chambers therein. Unlike the present invention, nucleic acid extraction and amplification all occur within a liquid phase and only the detection phase occurs in a lateral flow device.

A lateral flow device specifically for use with PCR is described in US 2001/0036634 A1. This application discloses an apparatus and method for performing nucleic acid amplification (via PCR), concentration and detection on a porous membrane. The specialised apparatus described is specifically designed for thermocycling incorporating a temperature block with stationary thermal zones onto which the lateral flow device is placed. The present invention differs from this disclosure in that the lateral flow device described herein is specifically designed for use with isothermal nucleic acid amplification techniques, whereby specific components of which may be tethered to the lateral flow matrix within the amplification region of the device. No specialised equipment is required. What is claimed in US 2001/0036634 A1 is an apparatus for thermocycling a reaction mixture in a continuous flow, specifically for the PCR.

The disclosure outlined herein provides for the complete analysis of a target nucleic acid, in an easy to use, fully integrated, self contained, disposable device. The device typically incorporates nucleic acid isolation / extraction, isothermal nucleic acid amplification, and

amplicon product detection all occurring within a porous lateral flow based matrix. The device disclosed has numerous applications and is also less prone to contamination issues due to its fully integrated design thereby requiring minimal manipulations.

The device described herein is a low cost, rapid, easy to use (no molecular biology skill required), sensitive, disposable, fully integrated unit, that has applications in clinical, veterinary, agricultural, food production, environmental, industrial and life sciences. One such application includes near patient testing, wherein due to its low complexity, patient samples may be analysed at the bedside in hospitals, or in the General Practitioners office.

#### **Summary of the Invention**

Disclosed is a method of, and apparatus for, testing for the presence of a nucleic acid sequence of interest (target), in a sample. Sample preparation, specific nucleic acid amplification and detection of amplicon all occur within a porous matrix, in an individual, disposable, self contained device.

The present invention allows for the (isolation and) detection of specific nucleic acid sequences whether composed of DNA or RNA. These sequences may be derived from, but not limited too, infectious disease agents, food spoilage organisms, human animal or plant sources. The subject of the invention has both proximal and distal termini and is composed of a series of porous or semi-permeable matrix/matrices where the various discrete reactions occur. The sample to be analysed is applied at the proximal terminus of the device and flows toward the distal terminal by capillary force. The analyte of interest is carried by this flow, through specific regions within the device that enable specific sequence amplification and detection to be performed, and typically also an initial nucleic acid extraction step.

In a first aspect of the invention provides a lateral flow assay device, the device comprising: a) a sample receiving zone for contacting the device with a sample to be tested; b) a porous carrier material in liquid communication with the sample receiving zone; c) a nucleic acid amplification zone located in liquid communication with the porous

carrier, and d) a detection zone for detecting the products, directly or indirectly, of the amplification reaction performed in the amplification zone, said detection zone being in liquid communication with the amplification zone.

In a second aspect the invention provides a method of performing an assay (e.g. detecting the presence of a nucleic acid of interest in a sample), the method comprising the steps of contacting the sample with an assay device in accordance with the first aspect of the invention, and determining the assay result.

The sample may be any sample of interest, such as a biological sample (e.g. blood, urine, sweat) or a sample of food or an environmental sample e.g. a water sample or a swab from a surface etc.

A preferred feature of the invention is that the porous carrier material serves to transport nucleic acid present within the sample applied to the sample receiving zone to the nucleic acid amplification zone where an amplification reaction takes place, said amplification reaction typically being dependent on the presence of nucleic acid of interest.

In a preferred embodiment, the device comprises a porous carrier, which may be unitary or a multi-component carrier, which serves to transport liquid from the sample receiving zone to the amplification zone and thence to the detection zone.

In one embodiment of the invention the device is composed in part of, a sample receiving and extraction zone (e.g. FTA matrix (Whatman Ltd.)), an amplification zone (e.g. GF/C matrix Whatman Ltd.), followed by a detection zone (e.g. nitrocellulose or nylon based membrane) (Figure 1). At the distal end of the device is an absorption zone or wick to receive fluids and provide capillary force. All of the zones are in fluid contact with one another and optionally may be attached to a backing (e.g. plastic sheet) to provide strength and rigidity. The membrane entities may optionally be encased in a protective casing provided with apertures for sample addition, reading of generated signal, etc. Sample of interest is introduced onto the sample receiving and extraction zone (located at the

proximal terminus) where extraction of target nucleic acid occurs. Extracted nucleic acid is released from the matrix (optionally by application of wash buffer) and flows by capillary force to the amplification zone containing amplification probes and enzymes. The amplification probes and enzymes may be releasably or immovably immobilized within the amplification pad, or a mixture thereof. Following amplification, the resulting amplicon interacts with labelled amplicon detection probe and the resulting reaction product is drawn through the porous membrane by carrier fluid to the detection zone. The detection zone includes a capture reagent (capable of binding to the labelled amplicon) which is directly or indirectly immobilized onto the membrane to form a test zone where amplicon is captured for visualisation or measurement, thereby indicating the presence or absence of the specific sequence of interest. The test zone can be in the form of a line, spot, etc., and the device can optionally include multiple test zones. It will be obvious to those skilled in the art methods by which amplicon can be captured on the test line. One or more control zones may also be included, preferably distal to the test line, to indicate successful amplification and completion of the assay.

For present purposes, a substance is considered releasably immobilised if it is released from a surface by contacting the surface with water or a dilute aqueous solution, such as sample buffer or other liquid which may be routinely employed in lateral flow assays.

In another embodiment, the proximal sample receiving zone can be a simple nitrocellulose or nylon based matrix (but not limited to these) whereupon pre-extracted nucleic acid (i.e. lysate) is applied, followed distally by amplification and detection zones. In this embodiment, the zone acts as a sample receiving zone (Figure 2).

In one embodiment, the amplification zone of the device may contain crosslinked moieties of isothermal nucleic acid amplification systems (Figure 3). These isothermal systems may be divided into nucleic acid target amplification systems and signal amplification systems. Examples of nucleic acid target amplification systems, are NASBA (US 5,130,238), TMA (US 5,399,491). Examples of signal amplification systems, are SMART/SPAR (WO 93/06240 and WO 99/37805 respectively) and Invader/Cleavase (US 5,846,717).

Target specific primers/probes associated with each of the above methods may be cross linked to the matrix of the amplification region pad (Figure 4) by a number of means obvious to those skilled in the art i.e. amine activated pad matrix cross linked to primers/probes via PITC (phenyldiisothiocyanate). The cross linked primers/probes capture the specific analyte in the amplification pad from the flow through and amplification ensues either by addition of remaining amplification components (enzymes, further primers/probes, etc.) within the carrier fluid or are located lyophilised within the amplification pad matrix.

In a desirable embodiment, the amplification zone may contain components of an isothermal nucleic acid signal amplification technology essentially outlined in WO 93/06240, WO 99/37806 and WO 01/09376 (Cytocell Ltd). In this particular embodiment the template probe is bound to the matrix by crosslinking agents such as, but not limited to, phenyl isothiocyanate or disuccinimidyl suberate (Figure 5). Other components comprising the isothermal amplification and detection systems are included in the carrier fluid, i.e. extension probe, linear amplification probe, labelled detection probe and amplification enzyme mix. In a further embodiment, the other components are moveably immobilized (in a dry form) within the amplification zone and rehydrated by the carrier fluid.

In an alternative, the amplification zone contains components of an isothermal nucleic acid signal amplification technology (e.g. as wherein in WO 93/06240, WO 99/37806 and WO 01/09376) the template probe is bound to latex particles (methods of which are well known in the art) (Figure 6). Other components comprising the isothermal amplification and detection systems may be included in the carrier fluid i.e. extension probe, linear amplification probe, labelled detection probe and amplification enzyme mix.

In a further embodiment, the other components are moveably immobilized (in a dry form) within the amplification zone and rehydrated by the carrier fluid.

For all of the above embodiments, the reaction can proceed in an essentially continuous manner (with reactants flowing from zone to zone) or a discontinuous manner, with the fluid flow being controllably interrupted or slowed between one or more zones to allow reaction products to accumulate before proceeding to the next zone. This controllable flow can be achieved by a variety of means, including (but not limited to) a physical switch, dissolvable barrier, restriction of capillary flow, etc.

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In a further embodiment, some or all of the components of nucleic acid amplification technologies and detection probes are not tethered to the matrix of the device but are applied within the carrier fluid at the proximal terminus (Figure 7). Control of flow rate, volume and gating, regulates that specific reactions occur in specified regions of the device at any single time e.g. amplification occurs in the amplification zone for the specified time required. Typically the carrier fluid will be distilled water or a suitable aqueous buffer such as TE buffer. Generally between 50 $\mu$ l to 2ml, preferably 100 $\mu$ l to 1ml, of carrier fluid will be applied, possibly in temporally spaced aliquots.

In a further embodiment, components of nucleic acid amplification technologies and detection probes are not tethered to the matrix of the device but are moveably immobilized within the matrix (e.g. by drying), and subsequently rehydrated by the carrier fluid. Control of flow rate, volume and gating, regulates that specific reactions occur in specified regions of the device at any single time e.g. amplification occurs in the amplification zone for the specified time required.

In a further embodiment, the detection zone on the device is composed of a test line and optionally a control line. The test line preferably comprises a crosslinked linear array of amplicon specific capture moieties that captures and concentrates the amplicon / detection probe hybrid for visualization or measurement. The control line is optional and advantageously contains a crosslinked linear array of amplicon specific capture moieties that captures and concentrates the desired amplicon. The control line is located distal to the test line and may be used, for example, to determine successful amplification has occurred

either by capturing the amplicon product of a control amplification, or, by the capturing of excess amplicon product of the specific analyte amplification.

A number of detection methods may be applied at the test and control lines. One preferred method is the use of gold colloid conjugated detection probe. This detection probe may be located within the carrier fluid or moveably immobilized within the Amplification region matrix. Upon synthesis of amplicon, hybridisation of labelled detection probe occurs followed by capture on the distally located test and / or control zones. Capture on these zones may be achieved by a number of means, including, but not limited to, an array of biotinylated capture probes tethered on a streptavidin test / control line, or by capture probes immobilized directly onto the membrane.

In a typical embodiment, reactions taking place on the device occur at a substantially constant temperature (determined by the amplification system used). The device therefore can be placed (but not limited to) on a hot block set at the desired temperature (Figure 8) or in an incubator set accordingly. The device therefore requires no specialised equipment.

In a further embodiment, when single stranded RNA targets are the analyte of choice, no extra thermal or chemical denaturant is required. When double stranded analytes are the analyte of choice then an initial thermal or chemical step is required in order to render the analyte single stranded for analysis by the device. If chemical denaturation is the method of choice then this method can be incorporated within the device chemistry and therefore will not require specialised equipment.

In a further embodiment, when FTA matrix is used at the proximal terminus of the device, sequence specific amplification may take occur within this region, i.e. within the FTA matrix, or, may be eluted from the matrix and carried by capillary action to a designated amplification region of the device. If amplification occurs within the nucleic acid extraction region, then the resulting device will only require a test line and, optionally, a control line region at its distal terminus (Figure 9).

### Example

#### Example 1. Assay for *E. coli* 23S rRNA

Described below is a device for the detection of *E. coli* 23S rRNA using one embodiment of the present invention in combination with the nucleic acid signal amplification technique as described in WO 99/37806.

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The device comprises a series of zones arranged linearly and in fluidic contact with one another for performance of the test (Figure 10). The zones include:-

#### 1. Sample Receiving and Extraction Zone

This zone comprises Whatman FTA

#### 2. Amplification Zone

This zone comprises a pad of Whatman GF/C material containing:-

(i) Template probe attached to 2um latex microparticles (amine modified and crosslinked via phenyldiisothiocyanate)

(ii) Extension probe

(iii) DNA polymerase

(iv) RNA polymerase

(v) dNTP's

(vi) rNTP's

(vii) Linear amplification probe

(viii) amplicon detection probe coupled to 40nm gold colloid, prepared by incubation of 40nm gold colloid (British BioCell) with thiol-capped probe for 1hour, followed by blocking excess binding sites with 1mg/ml BSA .

A mixture containing (i)-(viii) at the appropriate concentrations is prepared in transcription buffer also containing 5% w/v sucrose, and 50 $\mu$ l dispensed onto the pad. The pad is then dried by lyophilisation.

### 3. Detection Zone

This comprises a strip of nitrocellulose (HF135, Millipore) 5mm x 25mm with a transverse stripe of amplicon detection probe immobilized onto the membrane. The probe is suspended in 25mM phosphate buffer pH 7.0 containing 0.5mg/ml BSA and striped across the membrane. The nitrocellulose is then dried overnight at 21°C at a relative humidity of less than 20%.

### 4. Absorption Zone

A wick of Ahlstrom 222 (Ahlstrom) material, 5mm x 20mm

Components 1-4 are laminated onto adhesive-backed Mylar (Adhesives Research) to support the components and ensure their orientation. Fluidic contact is achieved by a 2mm overlap between adjacent components. For the amplification zone-detection zone junction, a 2mm gap is left between the parts of the pads adhered to the backing, and a non-adhered projection of the amplification zone pad of 5mm allowed to overlap the detection zone membrane. The projection and the detection zone are separated by a removable impervious plastic sheet that prevents fluid flow until the sheet is removed.

All of the components are placed within a moulded plastic housing which has an aperture at the proximal end for sample application, a window above the capture zone to visualize the result, and a slit through which the separation plastic sheet between amplification zone and detection zone projects. Internal projections in the housing at the sites of pad/membrane overlaps ensure tight fluid connections between adjacent components.

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To perform an assay, sample is added through the aperture onto the sample receiving and extraction zone and the device placed on a heated block at 41°C for lysis and release of nucleic acids. Carrier fluid (e.g. TE buffer) is then added from a dropper bottle, causing the extracted nucleic acid to migrate by capillary action to the amplification zone and resolubilise the reagents deposited therein. Typically between 50 $\mu$ l to 2ml of carrier fluid is added, preferably between 100 $\mu$ l and 1ml.

Following the resolubilisation of the amplification reagents within the amplification zone by the carrier fluid containing the released nucleic acid, generation of amplicon ensues if the target (23S rRNA from *E. coli*) sequence is present. As the amplicon is generated by the amplification reaction, it binds to the gold-labelled amplicon detection probe to form a labelled complex

Because the fluid connection to the detection zone is blocked by the removable plastic sheet, the amplicon generated, and the resulting labelled complex, accumulate in the amplification zone.

After 40mins, the separation sheet is removed, allowing liquid, together with labelled amplicon and free labelled amplicon detection probe to migrate into the detection zone. Any labelled amplicon present becomes bound to the immobilized amplicon detection probe stripe and forms a red-coloured line. The free labelled amplicon detection probe migrates past the capture line and is retained in the absorption zone.

The capture zone can be visualized through the window (or quantified by a reader), and a red line is indicative of presence of *E. coli* 23S rRNA target in the sample.

A variant would be to have the amplification zone pad projection sticking up above the detection zone membrane, and a button in the housing which is pushed down at the appropriate time to form the fluid connection (Figure 11).

### Figure Legends

#### Figure 1

- 1 Sample receiving and extraction zone e.g. FTA matrix

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- 2 Amplification zone with either matrix or particle bound amplification probes and enzymes
- 3 Nitrocellulose or nylon based matrix
- 4 Test line
- 5 Control line (optional)
- 6 Absorption zone

#### Figure 2

- 1 Sample receiving zone e.g. Nitrocellulose or nylon based matrix
- 2 Amplification zone
- 3 Nitrocellulose or nylon based matrix
- 4 Test Line
- 5 Control Line (optional)
- 6 Absorption zone

#### Figure 3

- 1 Sample receiving +/- extraction zone e.g. FTA matrix or nitrocellulose or nylon based matrix
- 2 Amplification zone containing cross linked moieties of isothermal nucleic acid amplification systems
- 3 Nitrocellulose or nylon based matrix
- 4 Test Line
- 5 Control Line (optional)
- 6 Absorption zone

Figure 4

- 1 Sample receiving +/- extraction zone e.g. FTA matrix or nitrocellulose or nylon based matrix
- 2 Target specific primers/probes are cross-linked to the matrix of the amplification zone
- 3 Nitrocellulose or nylon based matrix
- 4 Test Line
- 5 Control Line (optional)
- 6 Remaining amplification components lyophilised within the amplification pad matrix
- 7 Absorption Zone

Figure 5

- 1 Sample receiving +/- extraction zone e.g. FTA matrix or nitrocellulose or nylon based matrix
- 2 Template probe cross linked to amplification zone matrix
- 3 Nitrocellulose or nylon based matrix
- 4 Test Line
- 5 Control Line (optional)
- 6 Absorption zone

Figure 6

- 1 Sample receiving +/- extraction zone e.g. FTA matrix or nitrocellulose or nylon based matrix
- 2 Amplification zone
- 3 Nitrocellulose or nylon based matrix
- 4 Test Line
- 5 Control Line (optional)
- 6 Template probe bound to latex particles

7 Absorption zone

Figure 7

1 Sample receiving +/- extraction zone e.g. FTA matrix or nitrocellulose or nylon based matrix

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2 Amplification zone containing no tethered components of nucleic acid amplification technologies or detection probes

3 Nitrocellulose or nylon based matrix

4 Test Line

5 Control Line (optional)

6 Absorption zone

Figure 8

1 Sample receiving +/- extraction zone e.g. FTA matrix or nitrocellulose or nylon based matrix

2 Amplification zone

3 Nitrocellulose or nylon based matrix

4 Test Line

5 Control Line (optional)

6 Hot block set to required temperature for amplification system being used

7 Absorption zone

Figure 9

1 Sample receiving +/- extraction zone e.g. FTA matrix or nitrocellulose or nylon based matrix

2 Nitrocellulose or nylon based matrix

3 Test Line

4 Control Line (optional)

5 Absorption zone

Figure 10

- 1 Adhesive Mylar
- 2 Sample receiving and extraction zone
- 3 Amplification Zone
- 4 Removable plastic sheet
- 5 Capture line
- 6 Control line
- 7 Detection Zone
- 8 Absorption zone

Figure 11

- 1 Adhesive Mylar
- 2 Sample receiving and extraction zone
- 3 Amplification Zone
- 4 Section of housing
- 5 Plunger
- 6 Closure mechanism to retain fluid contact
- 7 Detection Zone
- 8 Absorption zone

Claims

1. A lateral flow assay device comprising: (a) a sample receiving zone for contacting the device with a sample to be tested; (b) a porous carrier material in liquid communication with the sample receiving zone; (c) a nucleic acid amplification zone in liquid communication with the porous carrier; and (d) a detection zone for detecting the product/s, directly or indirectly, of the amplification reaction performed in the amplification zone, said detection zone being in liquid communication with the amplification zone.
2. An assay device according to claim 1, wherein the amplification zone is used to perform an isothermal nucleic acid amplification reaction.
3. An assay device according to claim 1 or 2 wherein at least one of the components required to perform the amplification reaction is releasably immobilised on or in the porous carrier.
4. An assay device according to any one of claims 1, 2 or 3, wherein at least one of the components required to perform the amplification reaction is immobilized in the amplification zone.
5. An assay device according to any one of the preceding claims comprising an immobilised or releasably immobilised oligonucleotide.
6. An assay device according to any one of the preceding claims, wherein the device comprises (preferably in the sample receiving zone) reagents (such as FTA) suitable to cause extraction of nucleic acid from eukaryotic or prokaryotic cells present within the sample.

7. A method of performing an assay, the method comprising the steps of contacting a sample to be assayed with an assay device in accordance with any one of the preceding claims, and determining the assay results.
8. A method according to claim 7 wherein, following contact of the sample with the assay device, all of the assay steps are performed on or within a porous matrix of the device.
9. An assay kit comprising an assay device in accordance with any one of claims 1-6 claims and a supply of carrier fluid, which carrier fluid comprises at least one reagent required to perform the assay.
10. An assay device substantially as hereinbefore described and with reference to the accompanying drawings.
11. An assay method substantially as hereinbefore described and with reference to the accompanying drawings.

## ABSTRACT

Title: Lateral Flow Assay Device and Method

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Disclosed is a lateral flow assay device comprising: (a) a sample receiving zone for contacting the device with a sample to be tested; (b) a porous carrier material in liquid communication with the sample receiving zone; (c) a nucleic acid amplification zone in liquid communication with the porous carrier; and (d) a detection zone for detecting the product/s, directly or indirectly, of the amplification reaction performed in the amplification zone, said detection zone being in liquid communication with the amplification zone.

Fig. 1

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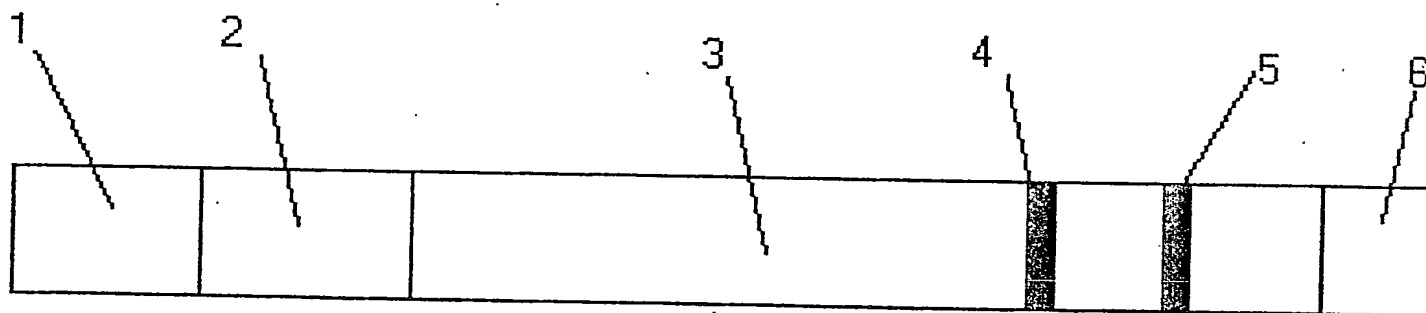


Fig. 2

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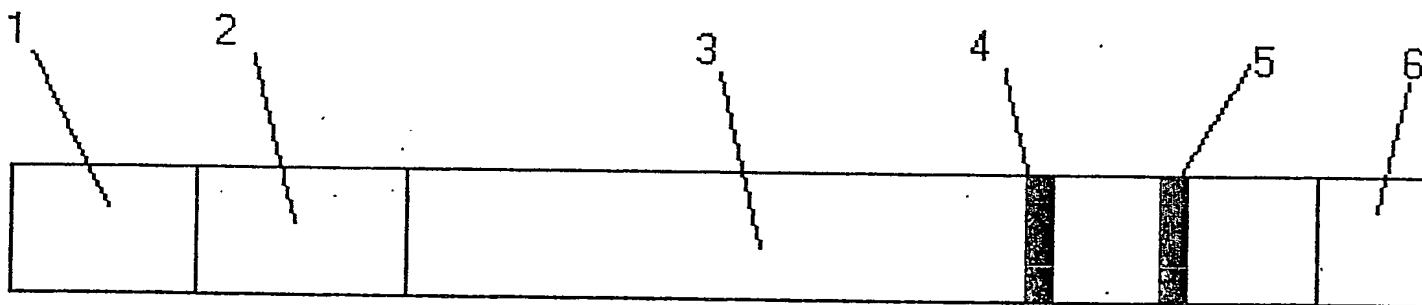
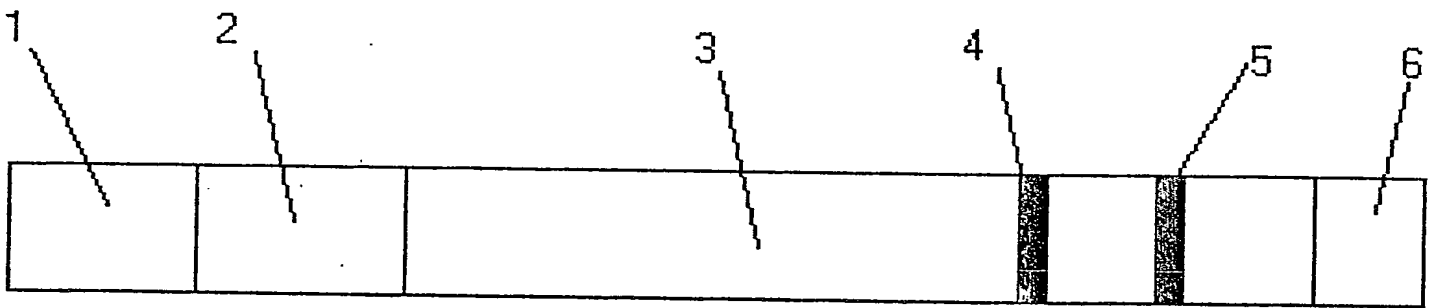


Fig. 3



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Fig. 4

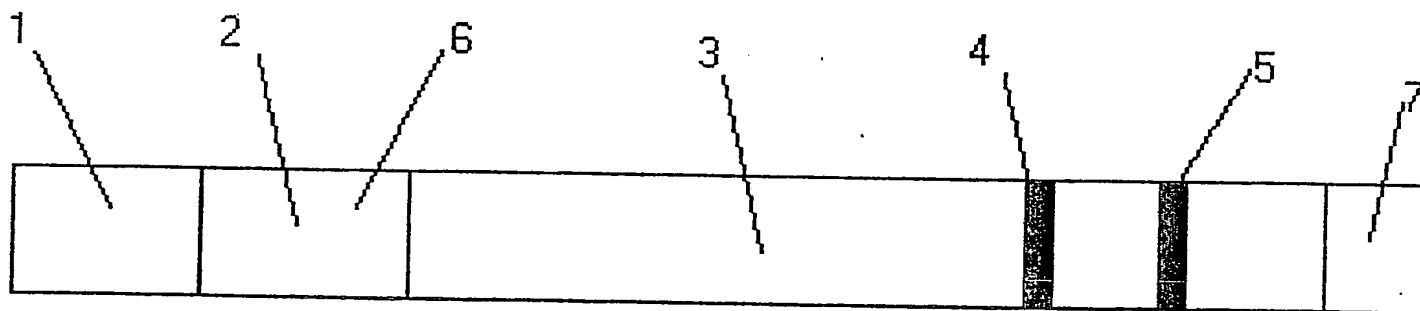
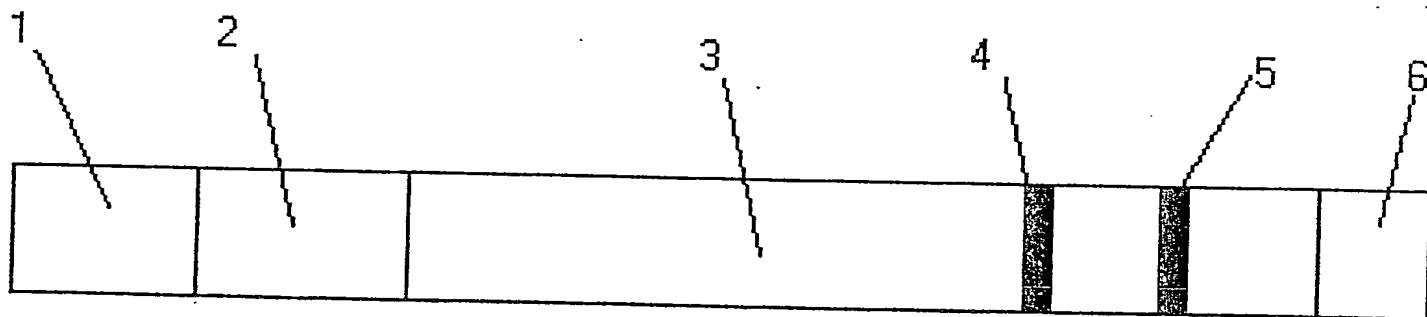


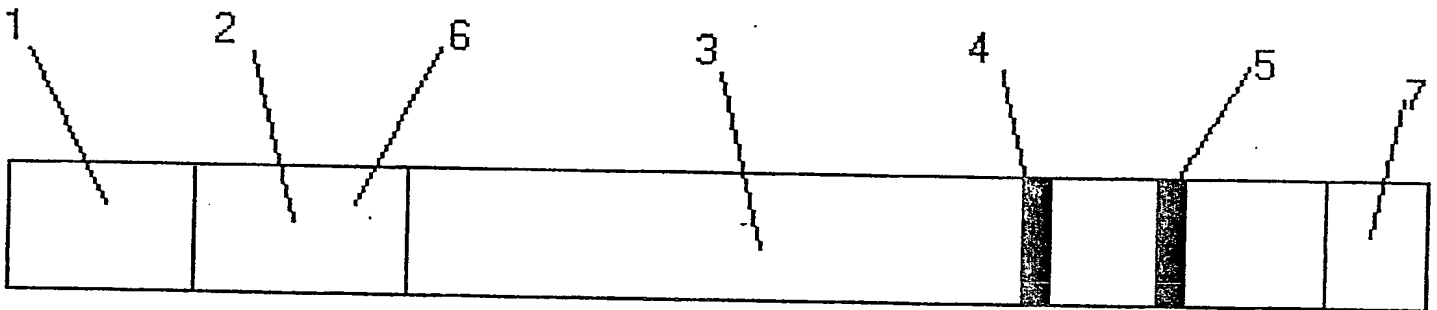
Fig. 5

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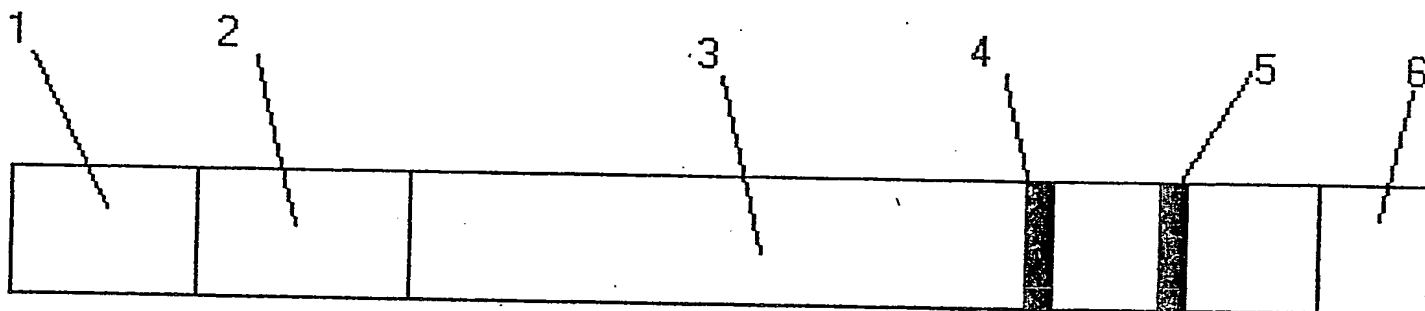
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Fig. 6



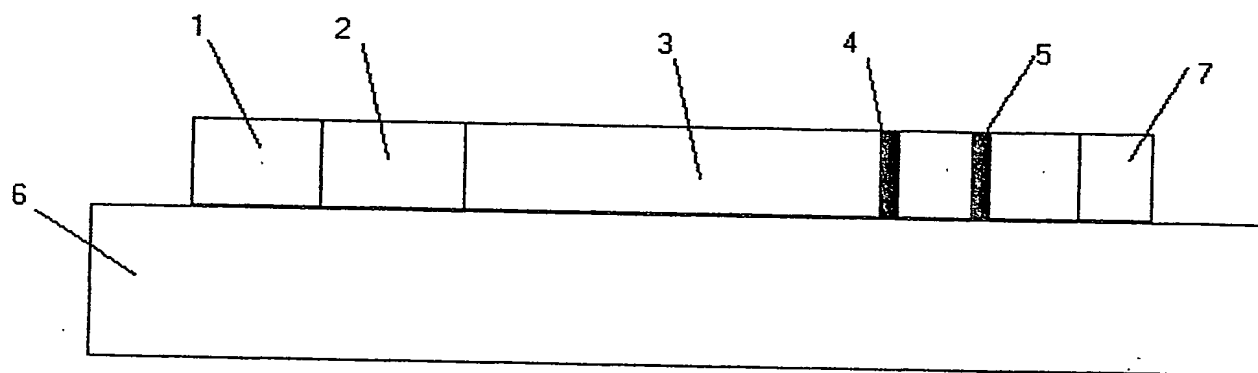
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Fig. 7



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Fig. 8



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Fig. 9

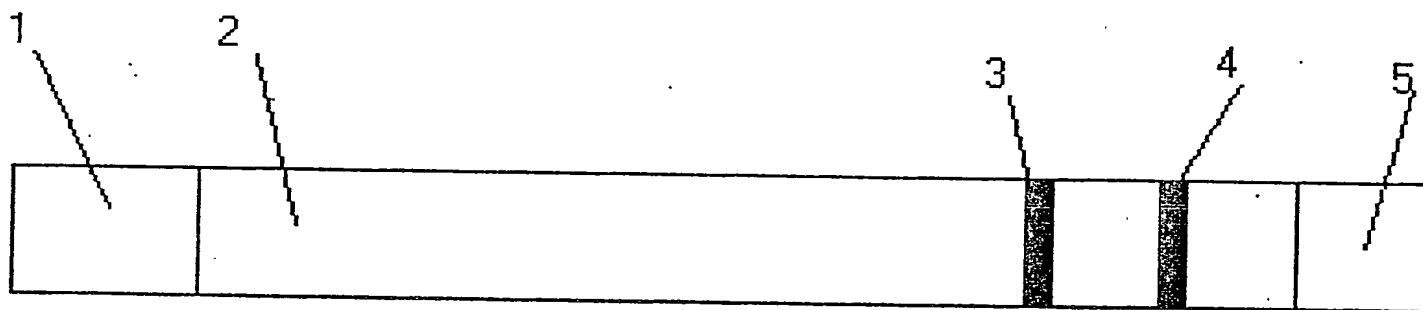


Fig. 10

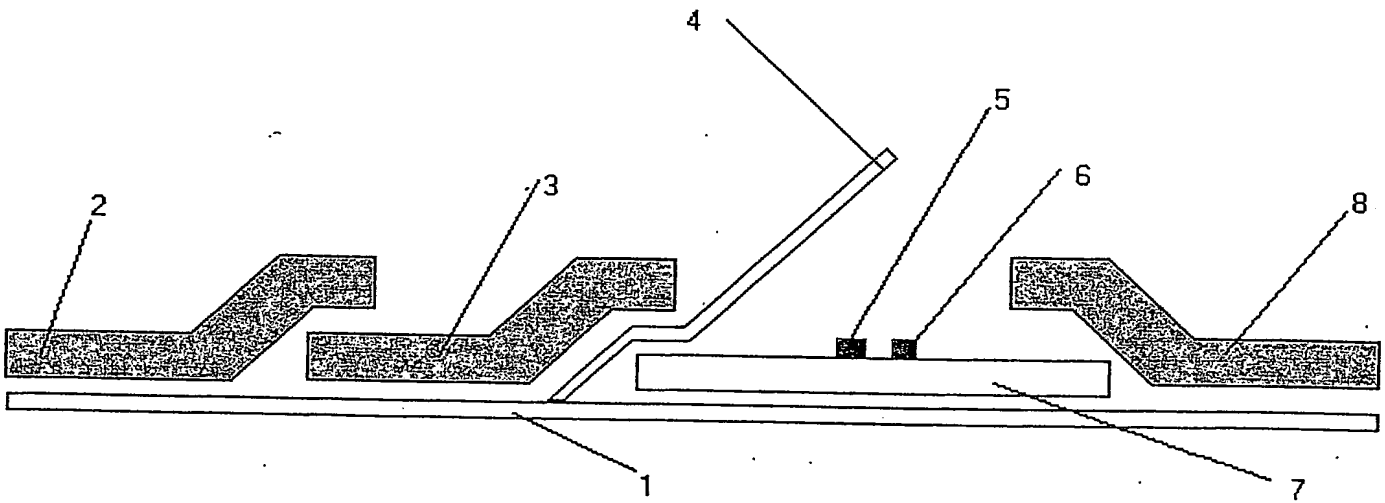
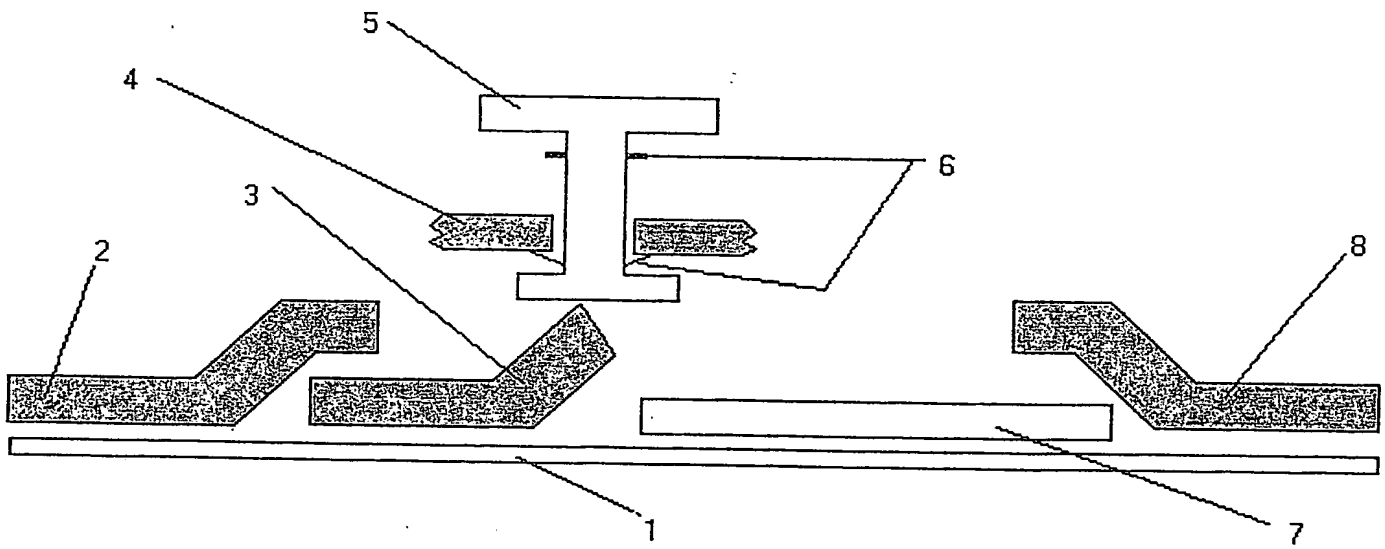


Fig. 11

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